II. Progress Report:

1. For some time, the principal focus of our work has been the development of methods for the purification of specific segments of bacterial DNA, and their artificial rejoining to produce new, genetically active arrangements. These microbial studies were intended to be a prototype for important applications in cell and cancer biology, pending the solution of formidable technical problems, and the clearing up of the biohazard policy issues that currently entail work with human DNA.

Our laboratory pioneered in the application of the 2-strand ligase of T4, which V. Sgaramella had shown here to be active on "flush-ended" DNA. However, incessant difficulties in producing such flush-cut segments led us to concentrate on the alternative systems using stagger-cut restriction endonucleases, which have indeed attracted explosive interest in dozens of laboratories. These enzymes have the special virtue of cleaving DNA at specific sites, and we have [as have many others now] used them for the purification of specific segments of DNA. As reported in [1], even as complex an ensemble as the total DNA of Bacillus subtilis has lent itself to separation by this approach (EcoRI endonuclease, followed by agarose gel electrophoresis.)

Now we are a) returning to the problems of T4-ligase
b) pursuing specific applications of the DNA-splicing methodologies
c) looking into the use of our current research as a testbed for computer-mechanized assistance to the planning of experimental work.

2. The work reported in detail in [1] was briefly summarized last year, and is the foundation for most of our continuing effort. It enables us to make consistent preparations of purified segments with specific genetic activity in the transforming system. These segments in turn have been spliced into various plasmids for amplification, and then tested for biological activity either in E. coli or in Bacillus subtilis.

Methods of using the plasmid DNA clones as reagent-probe-stains for electropherotograms have been developed, and they assure that homologous DNA has indeed been amplified. So far, however, we have no persuasive evidence of the biological activity of these segments -- but must also wonder whether we have correctly identified their genetic provenience. Our cautions -- perhaps even overstimulated by recent discussions about presumed biohazards of certain gene complexes -- have hampered rapid progress in this area by requiring the painstaking development of selective techniques that avoid the use of certain antibiotic-resistance markers, and these have come only slowly to hand (e.g. colicin resistance, which is sometimes less clearcut than the literature implies.)

At present we are systematically cataloguing the library of B. subtilis DNA segments so far amplified. Present methods stringently discriminate against the larger pieces that are of the most interest from a genetic standpoint; hence we are working out alternatives , like the deleted-lambda system, for application in this context.

We have also started to examine other endonucleases, e.g. from B. amyloliquefaciens, which segments B. subtilis at different sites, with a view to using overlapping segments for comprehensive mapping of the genome.

In work related to the flush-cut ligation, studies on the modification of virus P22 activity by exonucleases have been completed and mss. submitted to the Journal of Molecular Biology for publication. These have been provisionally accepted [3,4]. The most curious finding is the considerable AUGMENTATION of infectious activity of the viral DNA upon optimal terminal erosion, presumably by facilitating terminal circular sequence-pairing and closure. This is an important lead with respect to the early events of infection with purified DNA, and the decision of the race between the cell's defense mechanisms (including exonucleases )and the virus's initiation of its replication cycle.

In addition, we have independent evidence to corroborate the incorrectness of the random-permutation model of P22-DNA which has lain upon this field for many years.

Studies on B. subtilis transformation, involving heterogenous strains and their DNA hybrids, indicate that 1) sequence homology is the principal factor regulating the success of distant 'crosses', and 2), unlike transduction and crossing, host-restriction-modification systems play little if any role in transformation. The most likely explanation is the predominance of SINGLE-stranded DNA in transformation. [A practical concern is that singled-stranded VIRAL DNA, which is rarely found in nature, may be a particularly hazardous agent for xenobiotic exchanges when it does occur or may be artificially produced. As we do not really know whey even bacteriophage DNA is not (manifestly) infectious for man, no nugget of information of this kind is to be overlooked.]

Besides the earlier models for flush-cut DNA joining (mainly P22), we have also looked into the artificial production of such segments, and have found a B. subtilis enzyme that seems to work properly to this end. Accordingly, there is persuasive (but still preliminary) evidence that such segments can be inserted into plasmids and amplified.

As already indicated, the present policy turmoil about hybrid DNA has given pause about pursuing experiments with animal and human DNA, despite their enormous potential in many research and practical medical applications (see e.g., [5]), and we are therefore awaiting the resolution of these regulatory policies (and wondering how to pay for any new facilities that may be indicated) before moving towards such productive applications.

We have, however, decided to begin with the amplification of BACTERIAL T-RNA (in systems that must rank near the bottom of imaginable risk) as a prototype of similar studies to be done in due course with human material. These may be particularly relevant to cancer in the light of many reports on altered distribution of t-rna specificities in cancer cells. At the very least, amplified clones with specific t-dna sequences will provide histochemical reagents that should lend a new dimension of discrimination in the microscopic-pathological diagnosis of different cell types. We are just at the point of classifying which of the dna clones in our B. subtilis library contain the relevant t-rna information.

For some time I have been collaborating with Prof. E.A. Feigenbaum of the Computer Science Department on the development of computer programs that emulate some aspects of human scientific thinking. It is commomplace now to rely upon computers for tedious 'numbercrunching', data-banking and information retrieval applications; but the exaggerated claims for the future of artifical intelligence made about 15-20 years ago have not borne fruit, and have tended to put the field in some disrepute. Nevertheless, we have been able to make demonstrable progress in emulating some more creative aspects of scientific thinking, particularly in the formation of structural hypotheses -- until now mostly in the field of analytical organic chemistry [6]. This work has gone so well, that during the last year we have started a new pilot project to do much the same kind of work in the field of molecular genetics, with the mutually reinforcing advantages of being able to draw upon the 'real-life' activity of our research group in pursuing actual and difficult problems; and conversely that we might anticipate some reciprocal help in the solution of our own day-to-day problems in the laboratory. (This is just what happened in Dr. Djerassi's mass-spectrometry laboratory in connection with our other work.) This effort is getting enthusiatic attention from a considerable number of staff and students in Computer Science as well as from my own group. We are focussing on the computation of candidate PLANS for experiments to test hypotheses about the structure of DNA from a given source. The work so far seems also to be amenable to expressing (the more difficult) issues of the structural hypotheses themselves. If our previous effort with mass spectrometry is a relevant guide, it should take about five years for us to develop the data base and the sophistication in manipulating it to expect tangible help in our routine laboratory work. (This is of course contingent on funding support being sought from various sources.)

J. Lederberg

The undersigned agrees to accept responsibility for the scientific and thechnical conduct of the project and for provision of required progress reports if a grant is awarded as the result of this application.

Principal Invesitgator

26 January 1976
Date

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